PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Introducing the non-invasive prenatal test for trisomy 21 in Belgium: a cost-consequences analysis
AUTHORS	Neyt, Mattias; Hulstaert, Frank; Gyselaers, Wilfried

VERSION 1 - REVIEW

REVIEWER	Dick Oepkes Leiden University Medical Centre, Leiden, The Netherlands This reviewer has been involved in several studies on NIPT sponsored by Ariosa Diagnostics and Natera Inc.
REVIEW RETURNED	04-Jul-2014

GENERAL COMMENTS	A well performed and timely analysis, this is one of the major topics in obstetrics the coming 1-2 years. I cannot personally recalculate all numbers, but most of the assumptions and essential variables have been put is. Most limitations have been well addressed by the authors themselves. Although many of the numbers are based on information specific for Belgium, they will likely apply to many other countries. In particular, the real-wordl performance of the current combined test is often overestimated in modelling papers, or studies from expert-centres. With increasing use of NIPT, the quality of NT measurement is likely to decline further, not increase. This could be addressed by the authors. A figure that will immediately raise protest amongst readers, and therfore I suggest to change, is the risk of miscarriage following an invasive procedure of 1% in this manuscript. Quoting only Tabor 1986 is not acceptable anymore, there is for instance a Cochrane review with lower numbers. Also, the given cost of the combined test of 80 euros seems very cheap, in the Netherlands this is 154 euro. Two ther major discussion point in the international community at this moment are: what is the value of the first trimester anomaly scan (including NT), when trisomy 21,18 and 13 have already been detected by NIPT? This is not well researched, and may be disappointing. A datin gscan followe by a 20 week anomaly scan may be more cost-effective than keeping the 11-14 week scan. This needs to be addressed. Secondly, the reduction in number of invasive procedures with NIPT will lead to decrease of 'additional findings' in the current false positive group of 5%. Some colleagues find these additional findings, which vary based on which test is done (QF=PCR only, karyotyping, targeted or whole genome microarray), very important and stress that missing them will reduce quality of care. I personally find this a flawed argument, but it is repeatedly mentioned in recent papers by well-known experts, so it should also be addressed in this

	paper. Lastly, there is a consistent spelling error: 'life birth' should be 'live birth'. I recommend publishing after revision

REVIEWER	Amy Metcalfe University of Calgary, Canada
REVIEW RETURNED	07-Jul-2014

GENERAL COMMENTS	- This manuscript only focuses on Trisomy 21. While Trisomy 21 is the most common aneuploidy, different screening tests differ in their ability to detect other clinically relevant aneuploidies. At a minimum, some discussion of this is warranted - Some sensitivity analysis may be beneficial with regards to the assumptions used for the number of women undergoing an invasive test and terminating an affected pregnancy (see A Metcalfe 2013 Prenatal Diagnosis 33(5): 429-435 or S Chetty 2013 Prenatal Diagnosis 33(6): 542-546) Specific Comments - Some revision is needed for English grammar throughout the manuscript - The overall sensitivity of the trisomy 21 screening program referenced in the introduction is quite low. Presenting the separate sensitivity rates for women that present in the first trimester and second trimester, in addition to the overall rate, would be helpful for the reader to contextualize how this screening program relates to other programs - The authors assume no additional costs for counselling with NIPT – can you please describe when counselling is done in the current system and by whom? - In the reference case, the authors propose using NIPT as a second line test (followed by an amniocentesis if positive). Introducing a second test in the middle of this process will likely lead to a shift in the gestational age distribution at which terminations of pregnancy for Trisomy 21 are performed (undergoing NIPT and waiting for results will likely add approximately 7-14 days to the diagnostic process and might be the difference between a first trimester vs. second trimester abortion). Will there be a cost impact of shifting terminations of pregnancy to a later gestational age at which point in time more invasive procedures may be needed? - Operator experience has been shown to influence the procedure-related loss rate for amniocentesis. The availability of NIPT has been shown to reduce the number of amniocenteses performed. The authors may want to consider the possible clinical and cost implications of fewer amniocenteses, but a higher procedure-related loss rate in their sensitivity analyses This is a very interesting article that will likely have broader implications in the international community as other countries determine how to integrate NIPT into their current aneuploidy screening programs.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name Dick Oepkes
Institution and Country Leiden University Medical Centre, Leiden, The Netherlands
Please state any competing interests or state 'None declared': This reviewer has been involved in several studies on NIPT sponsored by Ariosa Diagnostics and Natera Inc.

A well performed and timely analysis, this is one of the major topics in obstetrics the coming 1-2 years. I cannot personally recalculate all numbers, but most of the assumptions and essential variables have been put is. Most limitations have been well addressed by the authors themselves. Although many of the numbers are based on information specific for Belgium, they will likely apply to many other countries. In particular, the real-world performance of the current combined test is often overestimated in modelling papers, or studies from expert-centres. With increasing use of NIPT, the quality of NT measurement is likely to decline further, not increase. This could be addressed by the authors.

- We agree with this and have mentioned the following in the discussion:
  "*Several experts have expressed their fear that the quality of NT will decline once NIPT is broadly introduced. The ultrasound should remain a key component of the prenatal screening process also after the introduction of NIPT in second or first line. Women with a foetal NT>3.5 mm (the 99th percentile) are directly (without use of biochemistry information) offered genetic counselling, diagnostic invasive testing and follow-up in keeping with international guidelines.*[19] *In such cases, there is a greater than 30% risk of chromosomal abnormalities, including but not limited to T21,*[17] *and other abnormalities such as heart defects.*[39 40]„
- In our conclusion we also mention "*Attention should be paid to further increase the quality of current screening with NT.*"

A figure that will immediately raise protest amongst readers, and therefore I suggest to change, is the risk of miscarriage following an invasive procedure of 1% in this manuscript. Quoting only Tabor 1986 is not acceptable anymore, there is for instance a Cochrane review with lower numbers.

- The Cochrane review (published in 2003 and assessed as up-to-date in June 2008) starts its discussion with the following: "*The best estimate of an 'excess' risk after second trimester amniocentesis comes from Tabor 1986. In a low-risk population with a background pregnancy loss of around 2%, a mid-trimester amniocentesis will increase this risk by another 1%.*"
  We added this in our text:
  "*Invasive testing carries a risk of membrane rupture with amniotic fluid leakage.*[13] *This may lead in about 1% of procedures to a hospitalization of about one week at a cost of €3515 and in about 1% to a procedure-related miscarriage. The latter is based on a Cochrane review which states that "the best estimate of an 'excess' risk after second trimester amniocentesis comes from Tabor 1986.*[14] *In a low-risk population with a background pregnancy loss of around 2%, a mid-trimester amniocentesis will increase this risk by another 1%".*[15] *This miscarriage rate may be more frequent after CVS compared with amniocentesis, and rates are expectedly lowerin experienced hands.*[14]„
- In Belgium, there are also many small centres performing this procedure. To make this more clear, we added the following in our discussion:
  "*The risk may thus be lower in the hands of experienced operators and higher in low–volume, less experienced centres. Currently, no required minimum volumes have been defined in Belgium and invasive testing is still performed in many small centres. Therefore we applied a 1% risk of procedure-related miscarriage after CVS or amniocentesis.*"
- In our conclusion we also write the following: "*As the number of invasive diagnostic tests will likely decrease, procedures should be centralized.*"
- Furthermore this 1% was varied in our probabilistic sensitivity analysis between 0.5% and 2%.

Also, the given cost of the combined test of 80 euros seems very cheap, in the Netherlands this is 154 euro.

- These are the costs for the health care payer in Belgium (see table 2: source NIHDI). In Belgium, the prices may be low, but the volumes are high. We remark that the price also excludes the costs for ultrasound. To make this more explicit, we added the following: "*Based on reimbursement data from NIHDI for the year 2011, excluding the 1.8% twin pregnancies,*

78,168 pregnant women participate in first trimester screening (€80.42 per activity) and another 21,451 in second trimester screening (€45.03 per activity). The fee for these activities is exclusive of the ultrasound but includes the counselling which is performed by the health care worker offering antenatal screening. NIPT is no replacement of the ultrasound screening and thus no incremental impact on ultrasound screening is included in the model."

Two ther major discussion point in the international community at this moment are: what is the value of the first trimester anomaly scan (including NT), when trisomy 21,18 and 13 have already been detected by NIPT? This is not well researched, and may be disappointing. A datin gscan followe by a 20 week anomaly scan may be more cost-effective than keeping the 11-14 week scan. This needs to be addressed.

- This option of including T18 and T13 in the NIPT and eliminating the first trimester US scan was not studied and was not suggested by the experts consulted.
- To take into account this remark and the following remark, we added the following paragraph in our discussion:
  "*When NIPT is compared with the current screening system, NIPT is clearly superior in terms of sensitivity and specificity for the detection of T21 and other types of trisomy. Nevertheless, the model focuses on the detection of T21 and does not take into account the effects of screening for trisomy 13 (T13) and 18 (T18). Among the aneuploidy forms, T21 has the highest birth prevalence rate.[20] Trisomy 18 occurs less frequently and T13 is rather rare and survival of neonates with T13 or T18 beyond the first days of life is rare.[21] The fetal fraction in T21 pregnancies is significantly higher compared with T13 and T18 pregnancies, which may help explain the higher sensitivity and specificity of NIPT for detecting T21.[22] More research is needed to evaluate the use of primary NIPT to detect trisomy 13 and 18 which may lead to more invasive tests because of false positive test results. If the current biochemical analyses are replaced by NIPT, the detection of some other chromosomal aberrations may be missed.[23] At present, the clinical importance is unclear as a NT>3.5mm will already pick up many of these abnormalities. This is of relevance, as keeping in place the biochemical screening in parallel with NIPT would lead to a much less pronounced drop in invasive testing with a different impact on both costs and effects of the NIPT scenarios modelled.*"

Secondly, the reduction in number of invasive procedures with NIPT will lead to decrease of 'additional findings' in the current false positive group of 5%. Some colleagues find these additional findings, which vary based on which test is done (QF=PCR only, karyotyping, targeted or whole genome microarray), very important and stress that missing them will reduce quality of care. I personally find this a flawed argument, but it is repeatedly mentioned in recent papers by well-known experts, so it should also be addressed in this paper.

- See the previous paragraph that is added in our discussion.

Lastly, there is a consistent spelling error: 'life birth' should be 'live birth'. I recommend publishing after revision

- This is corrected. A native speaker has corrected the article for grammar and spelling.

VERSION 2 – REVIEW

REVIEWER	Professor D. Oepkes Leiden University Medical Centre, The Netherlands I have conducted several clinical studies for which the costs were supported by companies providing NIPT, Ariosa Diagnostics and Natera Inc.
REVIEW RETURNED	24-Sep-2014

- The reviewer completed the checklist but made no further comments.

REVIEWER	Amy Metcalfe University of Calgary, Canada
REVIEW RETURNED	01-Oct-2014

GENERAL COMMENTS	The authors have adequately responded to the initial queries raised by the reviewers. I have no further comments at this time.